DISCUSSION

Diabetes Mellitus is a metabolic disorder that not only causes a decrease in efficiency of the pancreatic β cells to secrete insulin but also is accompanied by altered monoamine levels and their turnover rates in the CNS (Bhattacharya and Saraswathi, 1991, Garris, 1990, Lackovic et al., 1990). It is characterized by hyperphagia, polydypsia and activation of the hypothalamic pituitary axis (HPA) producing (Mohan Kumar, et al., 2003) a marked increase in food and water intake. Hyperglycemia is reported to be a major factor that damages the CNS monoaminergic activity as a result of neuronal degeneration in different regions of the brain. Onset of diabetes has been reported to inhibit the firing of dopaminergic neurons (Saller, 1984) with alteration in its metabolism. The magnitude and duration of dopamine signalling during diabetes is reported to be altered as a result of decreased activity of DA transporter (DAT) causing a low clearance of DA (Galli, et al., 2002; Figlewicz, et al., 1996; 2003). Hyperglycemia as a result of destruction in the pancreatic islets during diabetes is suggested to have an important role in the impairment of dopamine and other neurotransmitter functions.

Increased blood glucose and decreased body weight during diabetes is similar with previous reports as a result of the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod, et al., 1969). Hyperglycemia occurs as a result of increased glycogenolysis, decreased glycogenesis, increased gluconeogenesis, impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids via acetyl-CoA. During diabetes there is decrease in body weight as a result of altered metabolic function. Insulin treatment normalised the increased blood glucose level and decreased body weight to control values.

BRAIN MORPHOLOGICAL CHANGES DURING DIABETES

Microscopic sections from the brain regions using periodic acid stain in the CS, CC and HYPO revealed that there was an accumulation of glycogen granules in 14 day diabetic rats. Previous reports had only cited the accumulation of glycogen granules in the hypothalamus of 12 months diabetic rats (Bestetti & Rossi, 1980). Glycogen granules are reported to be absent or present in minimal quantities in the neuronal cytoplasm in normal conditions as these cells are reported to posses enzymes
involved in glycogen synthesis. Hyperglycemia is reported to cause an increased activity of these enzymes. Our results in the CS CC and HYPO of 14 day diabetic rats showed dense glycogen accumulation suggesting an increased formation of glycogen with destruction in the cells. This indicates the degenerative change in these brain regions which occurs in the early days of diabetes itself. Previous reports suggest that accumulation occurred in 12 months diabetic rats (Bestetti & Rossi, 1980). Insulin treatment mobilized the accumulated glycogen but did not completely improve the degeneration.

PANCREATIC MORPHOLOGICAL CHANGES DURING DIABETES

Pancreatic tissue section from control rats showed cells with distinct nuclei with hematoxylin and eosin stain. In the diabetic pancreatic section distorted nuclei and cells were observed. The damage in the pancreas as a result of diabetes resulted in hyperglycemia which we observed by the increase in blood glucose levels. Treatment with insulin brought a significantly elevated blood glucose level to control values. Onset of hyperglycemia caused severe progressive cell destruction. Streptozotocin induced diabetes is reported to cause marked degeneration in the pancreas (Bora & Srivastava, 1985; Ani, et al., 1996). Destruction in the pancreas is suggested to cause insulities as a result of lack of insulin. Insulin treatment reduced the damage to the tissue.

CENTRAL NERVOUS SYSTEM ALTERATIONS OF DOPAMINE AND HOMOVANILIC ACID DURING DIABETES

Several experimental models have been described which provide information on the etiology of IDDM. Sterptozotocin (STZ) is a toxic agent selective to pancreatic β-cells that induces IDDM by causing the β-cell destruction (Like & Rossini, 1976; Paik, et al., 1980).

In experimental models various doses of STZ have been used to induce diabetes and 65mg have been found to produce (Sumiyoshi, et al., 1996) maximum hyperglycemia. DA content in the striatum was affected by different doses of STZ. As the dose increased there was an increase in DA content and 65mg of STZ gave the maximum elevated DA content. When different doses of STZ were injected, an increase
in DA content was observed as a result of damage in the pancreatic islet population. The substantia nigra (SN) is an autonomic area in the central nervous system which plays an important role in controlling structure and activity of pancreatic islets. Lesions in the substantia nigra not only resulted in reduced size and number of islets cell populations but also decreased the content of insulin and glucagon in the pancreas (Smith & Davis, 1983). It has been established that central nervous system cell groups projecting into the pancreatic vagal motor neurons received inputs from the adrenergic, noradrenergic and serotoninergic neurons from the lower brain stem and a dopaminergic input from paraventricular nucleus of hypothalamus (Lowey, et al., 1994). Lesions in these brain regions are reported to affect the pancreatic islet cell population and growth. Similarly STZ damage of the islets disturbed the central dopaminergic connections altering the dopamine metabolism. Alteration in HVA has always been considered as an index of DA metabolism in the brain (Eisenhofer, et al., 1991; 1993; Roth, et al., 1983). In our results a significant decrease in HVA content on STZ injection indicates that the dopaminergic alterations are due to damages in the pancreatic islets. There was also a decrease in the conversion of DA to HVA as a result of STZ injection showing a decrease in the metabolism of dopamine.

In the time dependent study, DA content in the striatum began showing signs of increase 12 hrs after the injection of STZ and increased significantly at the end of 48 hrs. Dopamine content is reported to increase in the brain regions after 48 hrs of STZ injection (Onegova, et al., 1980). A concordant decrease in peripheral insulin levels after injection of STZ has also been reported which suggest the importance of the feedback mechanism between the metabolic disturbances as a result of insulin insufficiency and the changes in the monoamines level in the brain regions (Onegova, et al., 1980). The islet destruction by STZ has a role in causing stress to the brain by increasing the levels of DA during the onset of diabetes. The homovanillic acid content and the turnover ratio decreased as the content of DA increased. The decrease in HVA was observed 3 hrs after STZ injection when DA content did not show any change. This alteration in the DA metabolism indicates that damage in the dopaminergic system occurs as a result of the pancreatic islet destruction caused by the onset of diabetes. Thus central DA system and their alterations has a role in the etiology of diabetes.
Corpus Striatum

In the corpus striatum there was a significant increase in the dopamine content during diabetes. An increase in dopamine level in the striatum as a result of hyperglycemia attributes to the decreased release of dopamine (Lim & Lee, 1995). There was also a corresponding decrease in the HVA content during diabetes. The turnover ratio of DA to HVA also decreased during diabetes. Diabetes is observed to cause a decrease in dopamine metabolism (Kwok & Juorio, 1986). D-glucose is reported to depress the firing of dopamine containing neurons located in the substantia nigra by reducing the efflux of dopamine from the striatum (Saller, 1984). Similarly hyperglycemia during diabetes is reported to impair dopaminergic functions causing the accumulation of striatal DA inhibiting its efflux. Brain tyrosine concentration is decreased in diabetes (Frenstrom, 1983). Tyrosine is the precursor amino acid for the synthesis of dopamine. In diabetic condition the decrease in endogenous tyrosine concentration is reported to cause a reduction in the affinity of the enzyme tyrosine hydroxylase. The equilibrium in the tyrosine and tyrosine hydroxylase levels are reported to be much lower in diabetic rats. Diabetes is reported to cause a decrease in the accumulation of L-DOPA due to the inhibition of DOPA decarboxylase activity (Trulson & Himmel, 1983). The tyrosine hydroxylase enzyme mRNA expression in dopaminergic cells decreased during diabetes in the ventral tegmental area/substantia nigra compacta (VTA/SNc). Also, a decrease in the dopamine transporter protein (DAT) in the striatum is reported during diabetes which is also a primary factor for the elevation in the concentration of dopamine and decreased production of its metabolites (Figlewiz, et al., 1996).

The enzymes involved in the synthesis and metabolism of dopamine are reported to be decreased during diabetes (Trulson & Himmel, 1983). There was a significant increase in striatal DA content during diabetes which resulted in the notable reduction of HVA. D-glucose is reported to suppress the dopaminergic transmission and firing in the brain, lowering the DA metabolism decreasing the metabolite content. Saller, (1984) studied the changes in the HVA content at various time periods in the striatum and found that four days after the alloxan administration there was an elevation of HVA content which decreased 21 days later and declined consistently after 42 days. Our results showed
that the decrease in the level of striatal HVA observed in 14 day diabetic rats due to a
decrease in the metabolism of DA. Dopamine metabolites DOPAC and HVA
accumulation was decreased in STZ treated diabetic rats (Trulson and Himmel, 1983).
Administration of probenecid, an inhibitor of HVA, in STZ diabetic rats had no effect on
the metabolite accumulation that remained decreased, while in normal rats caused
significant accumulation of DOPAC and HVA.

Insulin therapy did not normalize the elevated dopamine levels in the striatum.
There are reports that insulin therapy during diabetes does not normalize the elevated
dopamine content and the reduced DA turnover rate (Sally, et al.1991). Insulin is
reported to be a key regulator in ameliorating the dopamine levels and its metabolism.
Reports gathered have shown that a complete recovery is not attained by insulin
treatment during diabetes (Gupta, et al., 1992; Bellush & Reid, 1991). We found that
insulin treatment for 14 days in diabetic rats had partial effect on the decreased DA
metabolism. Though insulin treatment did not reverse the elevated DA, it brought the
decreased HVA content to control levels during diabetes in the striatum. Previous reports
suggested that 4-6 weeks of insulin treatment caused a reversal of altered dopamine
content as insulin normalized its metabolism (Kwok & Juorio, 1986). Homovanillic acid
is implicated as an important marker for DA metabolism in the central and peripheral
nervous system (Eisenhofer, et al., 1991, 1993; Roth et al., 1983). The decreased HVA
with significantly low turnover ratio during diabetes is due to hyperglycemia. It has been
suggested that excessive production of glucose results in hyperglycemia during diabetes
impairing the metabolism of dopamine and other neurotransmitters (Girard, et al., 1995).
This hyperglycemic state during diabetes is due to the increased gluconeogenic pathway
which is physiologically less sensitive to the inhibition by insulin (Girard, et al., 1995).
Insulin induced dopamine release that was not fully effective in diabetic rats could also
be related to the peripheral insulin resistance exhibited by them (Cohen, et al., 1991).
Striatal dopamine release is affected by changing substantia nigra (SN) glucose levels.
This response may well reflect the known effect of glucose on K_{(ATP)} channel activity on
both SN DA neurons and GABA axon terminals in the substantia nigra. These
interactions could provide a mechanism whereby glucose modulates motor activity
involved in food intake (Levin, 2000). Diabetic rats manifested an altered behavioral and
neurochemical response suggesting a dysfunctional biosynthetic capacity for DA as a result of a decreased neurotransmission (Ahmad & Merali, 1989). The striatal dopamine content was elevated with a corresponding decrease in its metabolism during diabetes was only partially restored to control levels with insulin treatment in 14day diabetic rats.

**Hypothalamus**

Dopaminergic action is important in the regulation of the hypothalamic-pituitary hormone release. Hypothalamic dopamine content decreased during diabetes. Also, dopamine and its receptors are implicated in the satiety and hunger aspects and body weight maintenance. The central vagal connection with dopaminergic innervation is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while adrenergic and serotonergic innervations reach the pancreas through the brain stem (Smith & Davis, 1983; Lowey, et al., 1994). A decrease in DA in the hypothalamus during diabetes is caused due to the reduction in the low synthetic rate of dopamine as tyrosine levels decrease (Fernstrom, et al., 1983; Leu, et al., 1986). Altered dopamine is reported to affect the feeding pattern, as food intake is accompanied by DA release which differs significantly in the hypothalamus of obese and lean Zucker rats. The reduction in dopamine, norepinephrine and epinephrine levels in the hypothalamus suggests a low metabolism of monoamines (Bellush & Henley, 1990). They are responsible for the development of thermoregulatory deficits when exposed to cold environment (Leu, et al., 1986). Dopamine is considered as a hormone of the hypothalamus involved in the secretion of prolactin. It has an inhibitory effect on the release of prolactin from the anterior pituitary.

The decrease in dopamine content reduced the hypothalamic HVA with no alterations in turnover ratio of HVA from DA. This indicates a low synthesis of dopamine in the hypothalamus during diabetes. Insulin treatment for 14 days caused partial improvement of the DA and HVA content. The turnover ratio was near control values in all the groups. The decrease in DA and HVA content could be due to the depletion of dopamine resulting in a dysfunctional biosynthetic capacity for DA during diabetes (Ahmad & Merali, 1989; Merali, et al., 1988). This finding bear importance
since hypothalamus is reported to play a role in behavioral and physiological changes associated with diabetes.

Cerebral Cortex

Extracellular dopamine originates from DA and NE neurons in the prefrontal cortex (PFC). Recent reports suggested that extracellular DA release in the cortex depend on NE rather than DA innervation. In the cortex DA acts not only as NE precursor but also as co-transmitter (Gessa et al., 2001). The co-release of NE and DA seems to be controlled by α2 adrenergic receptors located on NE nerve terminals.

Cortical dopamine content increased with an increase in HVA content during diabetes. But the turnover rate of HVA from DA decreased during diabetes which indicates a decrease in the metabolism of dopamine turnover rate. Though there was a significant increase in both DA and HVA content, the metabolism of dopamine decreased as a result of hyperglycemia during diabetes. An increased DA and HVA content in the CC during diabetes is reported previously (Gupta, et al., 1992, Yan, et al., 1991). Cerebral cortical dopamine metabolism is reported to decrease because increased glucose during diabetes (Kwok & Juorio, 1986) affects the dopaminergic activities such as working, memory, and stress response (Tam & Roth., 1997). During diabetes a lack of tyrosine affect markedly the physiology and functions of these DA neurons. The overall deficit in the availability of the precursor amino acid tyrosine which has been previously reported has an influence in the functioning of DA neurons. Insulin treatment did not reverse the elevated DA and HVA to control values. This in compliance with previous reports that a complete recovery has never been attained by insulin therapy (Gupta, et al., 1992). The possible reason could be that during diabetes the alternative metabolic pathways supply glucose to provide energy. These pathways are reported to be resisting the inhibition by insulin causing only partial recovery (Girard, et al., 1995). This increased dopamine with a decreased turnover during diabetes in the cerebral cortex is associated with metabolic disturbance and behavioral changes. Efflux of DA in the prefrontal cortex is reported to stimulate hunger and food intake (Ahn & Phillips, 2002). Diabetes is marked by hyperphagia causing excessive food intake. Hyperglycemia causes the reuptake of DA into the brain cells which could possibly stimulate a
hyperosmolar state that results in dehydration causing polydypsia (Hirata, et al., 1992).

**Brain Stem**

Dopamine and HVA content in the brain stem decreased during diabetes correspondingly. A significant increase in the NE content in the brain stem from previous reports (Task, et al., 1992; Jackson, et al., 1997, 1999) suggested the decrease in DA could be because of the increased turnover to NE. This is important as the turnover to NE causes an increased sympathetic stimulation. This has important relevance in insulin secretion from the pancreatic islets as the increased sympathetic stimulation could inhibit the insulin secretion.

**ALTERATIONS IN DOPAMINE AND HVA CONTENT IN THE PLASMA, AND ADRENALS OF CONTROL, DIABETIC AND INSULIN TREATED RATS**

Dopamine concentration in the plasma during diabetes decreased significantly. This is in concurrence with earlier reports that showed that plasma DA decreased during diabetes (Chandrashekar-Reddi, et al., 1994). The plasma concentration of the DA is used as an indicator of central nervous system dopaminergic activity (Esler, et al., 1991). This decrease in plasma DA concentration indicates that diabetes causes an alteration in the overall dopaminergic function and activity. Peripheral plasma level of HVA, the deaminated and o-methylated metabolite of dopamine, is often used as an indicator of central nervous system dopaminergic activity (Esler, et al 1993). Our results show that HVA decreased significantly underlying the decreased metabolism of DA during diabetes. Regional HVA production is associated with the metabolism of dopamine in sympathetic nerves and it is at a rate which appears to be influenced by sympathetic nervous system. Also the turnover ratio of HVA from DA was also decreased during diabetes. The decreased DA with concordant decrease in HVA and the turnover ratio has immense importance as the plasma NE and EPI levels increased significantly. This increase in NE and EPI levels agrees with previous reports from our laboratory and others are due to central and peripheral increase in the sympathetic stimulation during diabetes (Jackson, et al., 1997; Chaouloff, et al., 1990a; Chandrashekar-Reddi, et al., 1994). In 14 day diabetic rats the turnover ratio of NE from DA also decreased as there was a significant decrease in DA. The activity of dopamine-β-hydroxylase (DBH) is reported
to be increased in blood from diabetic rats (Berkowitz & Head, 1978). Thus, the
increased activity of DBH causes an increased conversion of DA to NE which triggers
the sympathetic nervous system. Insulin treatment for 14 days showed improvements in
the concentrations of DA, NE, EPI and HVA. The turnover ratio of HVA from DA was
restored to control values reflecting changes in brain DA metabolism.

The dopamine and HVA contents in the adrenals decreased significantly
indicating an overall decrease in the metabolism of DA. Increased NE and EPI content in
the adrenal medulla during diabetes is observed as a result of the decreased dopamine
content. Most of the NE released is efficiently removed by neuronal and extraneuronal
uptake (Eisenhofer, et al., 1992). Evidences suggest that in the periphery DA serves not
only as a precursor for active compounds released from sympathetic nerves and the
adrenal medulla is suggested to act as an autocrine or paracrine regulator of local organ
function (Eisenhofer, et al., 1995). The increase in the sympathetic tone is because of the
increase in the NE and EPI levels in diabetic rats. Reports show a decrease in the NE
levels in the adrenal medulla during diabetes (Patel, et al., 1997). We observed a
decreased turnover of NE from DA during diabetes which could be as a result of
degeneration of the adrenals. A decreased turnover is being reported in the adrenal
medulla as a result of its degeneration during diabetes (Patel, et al., 1997). Also, it has
been suggested that the sympathetic tone is differentially altered in the peripheral tissues
and in the adrenals there is a decreased turnover during diabetes (Patel, et al., 1997).
Thus a decreased DA and HVA content with an increased NE and EPI levels during
diabetes increased the sympathetic stimulation. At the same time 14 day streptozotocin
diabetes indicated a lower turnover of NE from DA suggesting an overall damage in the
sympatho-adrenal system causing peripheral neuropathy.

Brain Dopamine receptor alterations during diabetes

Diabetes mellitus is often accompanied with emotional, behavioral, mood
disturbances and centrally mediated neurological complications (Salkovic & Lackovic,
1992). Striatal dopamine receptors were markedly decreased with no change in affinity
during diabetes with the accumulation of DA in the striatum and a decreased HVA
metabolism. Striatal dopamine firing during diabetes is decreased affecting
dopaminergic functions (Sailer, 1984). The decreased dopamine receptor density during diabetes is related to the decreased locomotor activity in STZ-induced diabetic rats (Kobayashi, et al., 1990; Shimomura, et al., 1990). This finding correlates with our present data suggesting that the disturbances in the central dopaminergic receptors during STZ-induced diabetes affects dopamine related functions. An increase in DA receptors is reported to cause an increased DA receptor sensitivity during diabetes (Lazovsky, et al., 1981) due to long term blockade of DA receptors or lesions in the striatal DA receptors. The firing of DA neurons projecting from the substantia nigra to the striatum is reported to be rapidly suppressed by hyperglycemia leading to the hypofunction of dopamine receptors (Sailer, 1984).

The decreased DA receptors during diabetes that we report in the striatum is a major cause in affecting dopamine related functions. There are hypothesis that suggests activities related to the functional capacities of DA receptors like stereotypy, ambulation, behaviour are diminished due to hyperglycemia (Lazovsky, et al., 1981). Also a decrease in DA receptors during diabetes may result in hyporesponsiveness (Kamei, et al., 1998). It is suggested that in alloxan treated rats with the onset of diabetes causes metabolic changes such as weight loss and dehydration are reported to occur which modify the DA metabolism (Omar, et al., 1985).

Insulin treatment effectively restored the decreased density to control levels but there was a decrease in the affinity of the receptors. The decrease in the affinity of the receptors during insulin treatment may be a compensatory mechanism in restoring the decreased dopaminergic function to normal state which is in compliance with previous reports.

The dopamine receptors in the hypothalamus did not alter in number during diabetes but there was a decrease in affinity in both Scatchard and displacement analysis. The Log (EC₅₀) value in diabetes increased with an increase in Ki suggesting a decrease in affinity state. Studies based on the reports from the anterior pituitary of alloxan treated diabetic rats showed no significant changes in the DA receptors and there was no modification in the binding affinity (Omar, et al., 1985). The pancreatic vagal motor neurons receive dopaminergic input from paraventricular nucleus of hypothalamus (Lowey, et al., 1994). This demonstrates the importance of CNS dopamine in the
pancreatic hormone secretion and in glucose homeostasis. Thus, hypothalamic dopamine receptors and their alterations are important during diabetes. Functions related to hypothalamus like increased water uptake and thermoregulatory deficits are suggestive of dopaminergic alterations directly or indirectly causing alterations in various autonomic, somatosensory, and motor neural functions of STZ-diabetic rats (Leu, et al., 1986).

The decrease in the DA receptor affinity during diabetes in the hypothalamus could be an important factor in the impairment of regulation of food intake and body weight. Our result in the hypothalamus suggests that decrease in the affinity of receptors during diabetes is linked to hyperglycemia. An alteration in the sensitivity of the receptors during diabetes has been previously reported causing a difference in the modulation of innervating DA systems. As the normal responses occurring in hypothalamic catecholamine metabolism after the consumption of food are modified by the presence of diabetes (Glanville & Anderson, 1986). Insulin treatment normalized the decreased affinity to control values. Insulin deficit and hyperglycemia affect hypothalamic DA receptor functions.

In the cerebral cortex the alterations in the DA receptors during diabetes showed an increase in the receptor density without any change in affinity. Dopamine in the cerebral cortex is thought to be involved in functions like motor functions, memory, and stress response (Tam & Roth, 1997). This indicates the long term blockade of DA receptors or damage in the cortical dopaminergic neurons as a result of hyperglycemia. Our studies have shown that [³H] DA binding is significantly increased in the cerebral cortex during diabetes. Increased DA receptor sensitivity and altered dopaminergic transmission has been implicated in the pathogenesis of schizophrenia (Lazovsky, et al., 1981). Our data suggest that elevated glucose causes the reduction of dopamine content leading to a compensatory increase in its receptors.

Thus the alterations in the dopamine receptors in the different brain regions had a differential effect during diabetes. Diabetes alters the sensitivity of the dopaminergic receptors and that altered response of the dopaminergic system could be indirectly involved in the modulation of nociception in diabetic rats possibly through the enhancement and/or deactivation of the endogenous met-enkephalinergic system (Kolta, et al., 2002). These alterations are of immense importance as chronic hyperglycemia
diminishes central dopaminergic function and increased dopamine sensitivity would be a compensatory adjustment to a reduced central dopaminergic activity.

**Brain dopamine D<sub>2</sub> receptor alterations during diabetes**

Striatal dopamine D<sub>2</sub> receptor density was significantly increased during diabetes. Previously \(^{3} \text{H}\) spiroperidol binding to dopamine D<sub>2</sub> receptors have been reported to be increased during diabetes (Trulson & Himmel 1983). Dopamine D<sub>2</sub> receptors were increased significantly during diabetes and insulin treatment did not reverse the increased number of receptors to control levels. Dopamine D<sub>2</sub> receptor antagonist \(^{3} \text{H}\) YM-09151-2 was also used for the receptor binding in the corpus striatum. The B<sub>max</sub> increased during diabetes and did not reverse during insulin treatment. This shows that during diabetes the dopamine D<sub>2</sub> receptors are significantly increased in the striatum and insulin treatment has only a partial effect in normalising the altered levels. The difference in the binding of \(^{3} \text{H}\) Spiperone and \(^{3} \text{H}\) YM-09151-2 is consistent with previous reports that \(^{3} \text{H}\) spiperone binds to dimers of the dopamine D<sub>2</sub> receptors and \(^{3} \text{H}\) YM-09151-2 binds to receptor monomers (Marzella, et al., 1997) inspite of both binding only to high affinity sites. Previous reports suggest that both the compounds have different affinities for the same dopamine D<sub>2</sub> receptors. The increased B<sub>max</sub> during diabetes is comparable to those changes observed after lesions of dopaminergic neurons or after chronic administration of dopamine receptor blockers. Excessive glucose or hyperglycemia is reported to deplete the dopamine metabolism and a decreased dopamine synthesis rate is suggestive to cause an increase in the receptor number of dopamine D<sub>2</sub> resulting in its increased number. This is reported to affect both the nigrostriatal and mesolimbic dopamine systems. Striatal dopamine D<sub>2</sub> receptor primarily represents a population of dopamine D<sub>2</sub> sites (Marzella, et al., 1997). Striatal dopamine D<sub>2</sub> receptors are reported to be involved in the modulation of morphine-induced antinociception in diabetic mouse (Kamei & Saitoh, 1996). During diabetes it has been documented that the sensitization of these receptors and their increased number results in a decreased locomotory and ambulatory activity (Kobayashi, et al., 1990; Shimomura, et al., 1990).
Dopamine through its dopamine D₁ receptor stimulates adenylyl cyclase and inhibits adenylyl cyclase activity through its dopamine D₂ receptors. Dopamine D₁ stimulate cAMP production was markedly increased in diabetic rats, whereas ability of dopamine D₂ receptor action to reduce cAMP formation was almost abolished during diabetes (Gorio, et al., 1989). An imbalance between Gs-proteins and Gi/Go protein mediated efficacy of Gs activity as a result of the loss of Gi/Go inhibitory functions has been found in the striatum and other tissues of diabetic animals (Salkovic & Lackovic, 1992). Dopamine D₂ receptors exert their function activating Gi proteins in the brain. Regulation of the inhibitory G protein-calcium channel complex involves pertussis toxin (PTX) sensitive and insensitive G proteins (Wiley, et al., 1998). Concomitantly with such transductional alteration detected in chronic diabetes, caused a marked increase of the striatal content of met-enkephalin, which is known to utilize Gi/Go proteins for inhibition of adenylyl cyclase. Diabetes causes the activation of mitogen activated protein kinase (MAP kinase) p38 as an early step in the signal pathway to dysfunction in experimental diabetic neuropathy (Agthong & Tomlinson, 2002). Increased content of dopamine and elevated B_max of dopamine D₂ receptors in the corpus striatum could cause a transductional defect in diabetic animals leading to diabetic neuropathy.

Diabetic neuropathy is the most common secondary complication of diabetes mellitus. Evaluation of the effect of levosulpiride, a selective antagonist for D₂ dopamine receptors, on the glycemic control of IDDM performed on 40 long-standing subjects with clinical signs of autonomic neuropathy and delayed gastric emptying improved glycemic control (Prando, et al., 1997). The effect of bromocriptine, a potent dopamine D₂ receptor agonist on intraocular pressure in diabetic patients with autonomic neuropathy revealed that it exerts an ocular hypotensive action through presynaptic dopamine receptors (Gale, et al., 1991).

Dopamine D₂ receptor gene expression increased in the striatum during diabetes as a result of the decreased transmission of dopamine. Hyperglycemia depresses the dopaminergic function and firing. Therefore a decreased dopaminergic activity is always suggested to increase the dopamine D₂ receptors. An increase in the expression of dopamine D₂ receptors gene results in the increased number.

Dopamine D₂ receptor cDNA is described to exist in two isoforms (short and
long) as a result of alternative splicing of the same gene that encodes for the receptor. They are represented as D2S and D2L. The longer form designated as D2L is the predominant form, although there is some variability among brain regions in the relative proportions of the two forms (Higgins, et al., 1991; Sealfon, et al., 1991; Todd, et al., 1996). Our RT-PCR data showed that the long D2L form expressed in all conditions and showed increase in expression during diabetes and insulin treatment. A lesion in the striatum is reported to increase the expression of dopamine D2L receptor gene (Zang, et al., 1994).

In the cerebral cortex also we report an increase in the Bmax of dopamine D2 receptors with out any change in the affinity during diabetes. It is suggested that chronic treatment with selective dopamine D1 or dopamine D2 receptor blockers induces a receptor-specific increase or decrease of DA receptors (Spano, et al., 1987). Therefore hyperglycemia is reported to bring about an increase in the number of dopamine D2 receptors during diabetes.

The dopamine neurons projecting to the prefrontal cortex (PFC) are thought to be involved in various motor and behavioural functions (Tam & Roth, 1997). This increased number of dopamine receptors could account for the behavioural supersensitivity to dopamine agonist as a result of damage in the dopamine functions (Cresse, et al., 1976). Diabetes mellitus is also reported to be one important factor for tardive dyskinesia caused by the chronic treatment with neuroleptic antipsychotic drugs which exert their action through the dopamine D2 receptors (Meltzer, et al., 1996). The increased binding of dopamine D2 receptors in the cerebral cortex with no change in affinity during diabetes has a relevance to the alterations in dopaminergic homeostasis affecting its function.

The dopamine D2 receptor mRNA in the cerebral cortex increased during diabetes and remained high after the treatment with insulin. Our receptor studies show an increase in the receptor number with a decrease in affinity. Therefore such a receptor expression pattern in the cerebral cortex may be due to differential translational regulation of the dopamine D2 receptor mRNA. Cortical dopamine D2 receptor expression has never been previously reported in diabetes. Dopamine D2L receptor mRNA expression was increased during diabetes in the cerebral cortex. Lesions in the corpus striatum is

Thus, dopamine D2 receptors during diabetes are increased in the striatum and cerebral cortex with an accumulation of DA. Dopamine D2 receptors are reported to regulate the release of dopamine from dopaminergic neurons originating in the ventral tegmental area as well as in the substantia nigra (Stoof, et al., 1987). Hyperglycemia during diabetes could damage the DA D2 receptors, decreasing the DA related functions in the striatum and other brain regions.

In the hypothalamus of diabetic rats the binding of $[^3H]$ YM-09151-2 to dopamine D2 receptors decreased significantly with an increase in affinity. The regional difference in the receptor status is relevant to the role which dopamine plays during various physiological and behavioural activites. Unis, et al., (1998) reported that $[^3H]$ YM-09151-2 binds to the dopamine D2 high affinity receptors. The decrease number of dopamine D2 receptors in the hypothalamus could result in the sensitization of its receptors leading to a shift into the higher affinity state. In the intra lateral hypothalamic area (Intra-LHA) blockade of dopamine D2 receptors by specific antagonist in tumor bearing (TB) and non tumor bearing (NTB) rats increased food intake indicating the involvement of dopamine D2 receptors in feeding mechanisms (Zhang, et al., 2001). Thus during diabetes the decrease in dopamine D2 receptor number or $B_{max}$ could disturb hypothalamic functions. Impairment of dopamine D2 receptor is an important factor that leads to hyperphagic and polydysopic condition as DA participates in regulating meal size (Oler, et al., 1997). Dopamine –acetylcholine (DA-Ach) interaction within the lateral hypothalamus (LH) is involved in the regulation of locomotion, feeding behaviour and reinforcement (Hoebel, et al., 2000, Baptista, et al., 1990). The cholinergic stimulation of these activities is regulated by DA through D2 receptors in the hypothalamus. Thus dopamine in the hypothalamus is related to sensory input, feeding reflexes, food reward or memory processes (Hernandez & Hoebel, 1988). In the hypothalamus co-administration of dopamine D1 and dopamine D2 agonists inhibit the feeding effect mediated by the action on neuropeptide Y (NPY) (Kuo, 2002). This is effective in the reduction of food intake in diabetic rats, revealing the efficiency of dopamine D1/ D2 agonist in the improvement of hyperphagia in diabetic animals. Dopamine D2 receptor
mRNA expression was in concordant with the receptor data showing a decrease in the expression of mRNA during diabetes and insulin treatment in diabetic rats caused an increase in the expression when to control levels. A decrease in dopamine D2 receptors are reported in obese Zucker rats which contribute to the specific feeding pattern in obese rats represented by an increased meal size and decreased meal number (Zhang, et al., 2002). Insulin is reported to regulate the re-uptake of catecholamine transporters. Intracerebroventricular injection of insulin is reported to cause an increased mRNA expression of DAT (Figlewicz, et al., 1994). We report an increased expression of dopamine D2 receptor mRNA during insulin treatment in diabetic rats. Our receptor analysis in the hypothalamus showed that insulin treatment did not fully restore the decreased receptor number and increased affinity to control levels. The increased expression during insulin treatment in the hypothalamus could be a mechanism to normalise the decreased number to control levels. Thus, decrease in dopamine D2 receptors in the hypothalamus due to lesions rising as a result of hyperglycemia.

Brain stem is an important part of the brain in monitoring the glucose status and the regulation of feeding (Guillod, et al., 2003). In the brain stem also there was a significant decrease in the dopamine D2 receptor density with a decrease in the affinity resulting in an overall down regulation of the receptor. Brain stem dopamine D2 receptors have never been reported previously. During diabetes the significant increase in NE and EPI (Tasaka, et al, 1992; Jackson, et al., 1997; 1999) could bind to α2 adrenergic receptors increasing the sympathetic nerve discharge could inhibit insulin secretion from the pancreatic islets. From our data we suggest that the increased activation of sympathetic stimulation during diabetes as a result of increased NE and EPI is because of a decreased DA content in the brain stem with a decrease in the down regulation of dopamine D2 receptors. This down regulation of dopamine D2 receptors in the brain stem could have a possible role in the regulation of insulin secretion by releasing EPI and NE from the adrenal medulla that leads to the inhibition of insulin secretion in the pancreas. In the brain stem there was a decrease in the expression of dopamine D2 receptor mRNA as a result of diabetes which increased further on insulin treatment. This could be due to the differential transcriptional regulation during diabetes. Insulin treatment brought the Bmax to control values but there was an increase in affinity...
of the receptors. Insulin is reported to have a modulatory effect on CNS dopamine and insulin injection is suggested to cause an increase in dopaminergic function (Figlewicz, et al., 1996, 1994). It has been reported that damages in the brain can cause an alterations in the expression of the dopamine D2L isoform which is expressed in the in vivo condition (Higgins et al., 1991; Sealfon, et al., 1991; Todd et al., 1996).

**Hypothalamic-pituitary-axis and dopaminergic functions during diabetes**

The hypothalamus is involved in the monitoring of glucose status and the regulation of feeding and hunger (Guillod, et al., 2003). Dopamine content during diabetes decreased in the hypothalamus with a decrease the number of dopamine D2 receptors and corresponding decrease in the dopamine D2 receptor gene expression. Diabetes activates the HPA axis producing (Mohan Kumar, et al., 2003) a marked increase in food intake and water intake which is completely reversed by insulin treatment. Dopaminergic neurons are the direct targets for insulin action which participate in the reward seeking behaviour (Figlewicz, et al., 2003). Therefore during diabetes the decreased availability of dopamine could affect these functions. We report a decreased dopamine content and dopamine D2 receptors in the hypothalamus during diabetes. This could decrease dopaminergic signalling in the hypothalamus. Diabetes is reported to decrease the dopamine transporter thus reducing the dopaminergic signaling affecting dopamine related functions (Galli, et al., 2002). Dopamine D2 receptor disruption is reported to impair body growth and the somatotroph population (Becu-Villalobos, et al., 2002). We report a decreased $b_{max}$ of dopamine D2 receptor during diabetes which could impair the body growth. Thus, a decreased dopamine with a decreased dopamine D2 receptor expression and receptor number in the hypothalamus as a result of diabetes affects the metabolic functions of the hypothalamus.

**Dopaminergic alteration during diabetes in the pancreas and its significance in glucose metabolism**

Pancreatic islets are sited to contain dopamine in the secretory granules along with serotonin and calcium (Ahren & Lundquist, 1985). There was a significant decrease in the turnover ratio of HVA from DA in the pancreas during diabetes. Pancreas is an important source of non-neuronal dopamine in the body and that this dopamine has a role
in protecting the intestinal mucosa (Hoffman, *et al.*, 1996). Early sympathetic islet neuropathy (eSIN) is reported to occur selectively in the islet during diabetes in diabetic rats (Taborsky, *et al.*, 2002) as a result of monoamine alterations. Increased NE in the islets could possibly be because of the increased uptake and decreased degradation. The increased NE and EPI content with a decreased DA and HVA levels in the adrenal medulla and plasma during diabetes was observed. Most of the NE released is efficiently removed by neuronal and extraneuronal uptake (Eisenhofer *et al.*, 1992). Evidences suggest that in the periphery DA serves not only as a precursor for active compounds released from sympathetic nerves and the adrenal medulla but also is suggested to act as an autocrine/paracrine regulator of local organ function (Eisenhofer, *et al.*, 1995). The central nervous system cell groups project into the pancreatic vagal motor neurons receive adrenergic, noradrenergic and serotonergic inputs from the lower brain stem and dopaminergic input from paraventricular nucleus of hypothalamus demonstrating the importance of CNS dopamine in the pancreatic hormone secretion and glucose homeostasis (Lowey, *et al.*, 1994). Thus, dopamine content in the pancreas showed marked decrease with a decrease in its metabolism. This could be related to the sympathetic tone that is increased during diabetes as a result of an increased NE and EPI levels in the plasma, pancreas and the adrenals. The metabolic clearance rate of DBH is a major factor accounting for the increase in DBH activity in the streptozotocin-diabetic rat (Stolk JM, *et al.*, 1982, (Watanabe & Nagatsu, 1991). Hyperglycemia could possibly decrease the metabolism of DA present in the pancreatic secretory granules that would affect the pancreatic islet function. As findings suggest that an endogenous alterations in these hypothalamic monoamines may contribute to islet dysfunction, which is characteristics of diabetes (Cincotta, *et al.*, 1999).

The dopamine receptors showed an increased $B_{\text{max}}$ during with a decrease in its affinity in the pancreatic islets. This was confirmed by both Scatchard and displacement analysis. The decreased dopamine content and metabolism as a result of hyperglycemia could be a cause of the increase in the receptor parameters in the pancreatic islets causing the sensitization of these receptors. Pancreatic dopamine receptors have not been a focus of studies till date except for a few reports (Imamura, *et al.*, 1990). It has been observed that the sympathetic alpha receptor and dopamine $D_1$ was distributed on the B-cells, the

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sympathetic β2 receptors on the D-cell and the dopamine D2 on the varicosity of the sympathetic β2 neuron. Dopamine binding sites are in the pancreatic arcinar cells are suggested to be receptors that mediate the action of dopamine on cAMP accumulation (Ribet, et al., 1986). Studies in the past indicate that dopamine directly affects pancreatic islet B and D cell function. Dopamine suppresses somatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through an alpha adrenergic mechanism and stimulates glucagon release through a β adrenergic mechanism (Gerich, et al., 1982). Dopamine acts on specific dopamine receptors related to the exocrine pancreatic secretion and sulpiride was found to be a potent dopamine antagonist in the canine exocrine pancreas (Honda, et al., 1980). The increased dopamine receptors in the pancreatic islets could be as a result of the decreased dopamine turnover and as a result of the increased adrenergic activity that is damaged during diabetes.

Binding of [3H] YM-09151-2 for dopamine D2 receptors in the pancreatic islets decreased significantly with an increase in affinity. The Log (EC50) value during diabetes decreased with an increase in affinity (Table 32, Fig.-34). This is similar to what we obtained in the hypothalamus for dopamine D2 receptor. The dopamine D2 receptors in the pancreatic islets demonstrate a down regulation in receptor number with an increase in affinity. The damage caused as a result of hyperglycemia with an increased sympathetic stimulation in the pancreatic islets could be a possible cause for the decreased activity of dopamine D2 receptors in the pancreatic islets during diabetes. Dopamine D2 receptor alterations will possibly have an effect on the pancreatic islet population causing a decrease in insulin secretion.

**Alterations in the dopamine and dopamine D2 receptors during diabetes**

Dopamine receptors decreased significantly in the striatum while the dopamine D2 receptors increased in the number. In the cerebral cortex, dopamine DA and dopamine D2 receptors showed an increase and the affinity of DA receptors decreased. The hypothalamic dopamine DA receptor number did not alter but there was a decrease in affinity while the dopamine D2 receptors decreased with an increase in affinity. In the brain stem the dopamine D2 receptors decreased with a decrease in affinity showing an
overall down regulation. Thus from our study we conclude that the altered dopamine receptors and dopamine D₂ receptors binding observed in brain region of diabetic rats increases the sympathetic stimulation. Altered dopamine is reported to mediate an increased sympathetic nerve discharge (Bauhelal & Mir, 1993). During diabetes in the pancreatic islets the decrease in dopamine and its turn over increased dopamine receptors. The dopamine D₂ receptors decreased with an increase in affinity. The overall decrease in the dopaminergic function is as a result of the increased EPI and NE release from the adrenals into circulation and pancreas could lead to an inhibition of insulin release.

**Effect of norepinephrine on dopamine uptake in the pancreatic islets.**

Dopamine is stored in the secretory granules of the pancreatic islets along with serotonin and calcium (Ahren & Lundquist, 1985). The uptake studies using [³H]DA in the pancreatic islets revealed that DA uptake was maximum in the presence of 10⁻⁴ [³H]DA in both the concentrations of glucose (4 & 20mM) and the uptake decreased with a decrease in DA concentrations. These results indicate that in the presence of glucose there is an uptake of DA into the pancreatic islets. At high concentrations DA is always taken up into the pancreatic islets in both the concentrations of glucose. This could have an implication in insulin secretion as high concentrations of DA in the presence of glucose stimuli causes a reduction in insulin secretion. Dopamine is reported to modulate insulin secretion in the pancreatic islets with changes in calcium efflux (Carpinellie, et al., 1994). Possibly a high DA concentration in the islets is essential in maintaining the equilibrium during insulin secretion. The function of islet β cells is controlled by a glucose sensor that operates at physiological glucose concentrations and acts in synergy with signals originating from hypothalamic neurons. Evidence exists that the extra pancreatic cells producing and secreting these neuroendocrine signals also exhibit a glucose sensor and an ability to integrate nutrient and neuro hormonal messages (Pipeleers, et al., 2001). From our uptake studies in the pancreatic islets we suggest that the DA is involved in glucose induced insulin secretion.

We observed that NE at low concentration did not have any effect on the [³H]DA uptake while at high concentration inhibited the uptake of DA in the presence of 4mM and 20mM glucose. Thus, high concentration of NE blocked the uptake of DA into
The pancreatic islets and this could affect the role of DA in glucose induced insulin secretion. Increased NE level is reported to inhibit the pancreatic islet function (Sheen, et al., 2001). The blockade of DA into the pancreatic islets by NE is as a result of its increased uptake by neuronal and extraneuronal tissue which causes the inhibition of insulin secretion. The following points are inferred from our uptake studies:

1) Dopamine transport into the islets requires glucose and high concentrations of DA prevent glucose transport into the pancreatic islets.

2) Dopamine in the secretory granules of the pancreatic islets could be one of the possible elements that operate at physiological glucose concentrations. It acts in synergy with signals that integrate messages originating from hypothalamic neurons and pancreas and damage to this could be a possible cause of the inhibition of insulin secretion during diabetes.

**Effect of dopamine on glucose induced insulin secretion in vitro**

Dopamine in the presence of glucose had a dose dependent effect on insulin secretion. We observed that low concentrations of DA increased glucose (20mM) induced insulin secretion while high concentration caused the maximum inhibition. Dopamine at high concentrations reported to inhibit insulin secretion from the islets (Carpinelli, et al., 1994). Also, high concentrations of norepinephrine, dopamine, and serotonin in the pancreatic islets are reported to decrease glucose-stimulated insulin secretion (Feldman, et al., 1980).

We observed that butaclamol, antagonist for dopamine receptors blocked the inhibitory and the stimulatory effect of DA in the pancreatic islets mediated. The addition of sulpiride a potent dopamine D2 receptor antagonist to the pancreatic islets effectively blocked the dopaminergic action on insulin secretion. In previous studies from our laboratory reported that addition of forskolin an activator of cAMP resulted in overcoming the effect of DA on insulin secretion (Abraham, 1998).

Dopamine D2 receptors agonists bromocriptine (BRC) and 7-OH-DPAT were used to study their effect on glucose induced insulin secretion in the pancreatic islets in vitro. Bromocriptine a potent dopamine D2 agonist at low concentrations stimulated glucose induced insulin secretion in the presence of 20mM glucose while in high
concentrations had an inhibitory effect. The stimulation by BRC at its low concentration was not as effective in the presence of 4mM glucose. It has been reported previously that BRC treatment in hyperglycemic state had a strong stimulatory response to insulin secretion (Oliveira, et al., 1998). The agonists of dopamine by acting through the neuroendocrine system improves peripheral energy metabolism and impaired islet function. (Lang, et al., 1998). 7-OH DPAT showed an inhibitory effect on glucose induced insulin secretion. Previous reports suggest that 7-OH DPAT induced hyperglycemia decreased insulin secretion (Hillegaart, et al., 1996). In vitro studies confirmed the stimulatory role of dopamine D2 receptors on insulin secretion.

Norepinephrine is reported to have an antagonist effect on insulin secretion in the pancreatic islets (Porte, et al., 1967). Low concentration of NE did not affect the stimulatory effect of DA on insulin secretion while high concentrations of NE was found to be inhibitory. It has been previously reported that high concentrations of NE inhibited the glucose induced insulin secretion (Zren, et al., 1980). During diabetes there is an increased neuronal and extra neuronal uptake of NE that increases the sympathetic stimulation (Eisenhofer, et al., 1992). This blocks the insulin secretion as the increased sympathetic tone elevates peripheral insulin resistance and hyperglycemia. Thus, our in vitro results show that increased concentrations of NE blocked the stimulatory effect of low concentrations DA.

Our in vitro studies show that low concentration of dopamine is necessary in the stimulation of insulin by glucose and this is mediated through the dopamine D2 receptors in the pancreas. We report a decrease in the metabolism of DA with differential alterations in the dopamine DA and D2 receptors in the brain and pancreas during diabetes. The decreased dopaminergic tone with a high turnover to NE and EPI results in an increased sympathetic stimulation decreasing the β-cell responsiveness to parasympathetic stimulation to secrete insulin. The increased NE not only blocks the uptake of DA but also inhibits its stimulatory effect on insulin secretion. Dopaminergic dysfunction is an important factor during diabetes which not only affects the central functions but also is a cause for the decreased insulin secretion from the pancreatic islets.